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CNS EFFECTS OF HEAVY PARTICLE IRRADIATION IN SPACE: BEHAVIORAL IMPLICATIONS

J. A. Joseph*, S. Erat*, and B. M. Rabin**

**USDA Human Nutrition Research Center on Aging, Boston, MA 02111*
***University of Maryland Baltimore County, Baltimore, MD 21228*

Research from several sources indicates that young (3 mo) rats exposed to heavy particle irradiation (^{56}Fe irradiation) produces changes in motor behavior as well as alterations in neuronal transmission similar to those seen in aged (22-24 mo) rats . These changes are specific to neuronal systems that are affected by aging. Since ^{56}Fe particles make up approximately 1-2% of cosmic rays, these findings suggest that the neuronal effects of heavy particle irradiation on long-term space flights may be significant, and may even supercede subsequent mutagenic effects in their mission capabilities. It is suggested that among other methods, it may be possible to utilize nutritional modification procedures to offset the putative delterious effects of these particles in space. Published by Elsevier Science Ltd on behalf of COSPAR

INTRODUCTION

In examining the literature concerned with the biological effects of high energy charged particles (HZE's) from cosmic irradiation exposure on long-term space flights, it is surprising how little attention has been given to possible neurological sequelae that might result from such exposures. Although genetic factors such as weightlessness and genetic aberrations etc. have received extensive study during previous orbital missions, the potential health hazards of extended space-flights with respect to brain and behavior have received minimal study (NCRP, 1989). For example, the putative effects on motor behavioral performance have only recently been addressed in a few papers (see below).

As is well known, there are a variety of sources of HZE exposure in space that could occur for example: a) during major solar events in which the flux of heavy particles can increase rapidly by three or four orders of magnitude above the galactic cosmic-ray background (Stassinopoulos, 1988) b) from cosmic irradiation, wherein heavy charged particles, with energies to 10 GeV/nucleon, constitute about 1-2% of galactic cosmic radiation. The potential for HZE particles to alter central neuronal functioning and behavioral performance becomes increasingly important when one considers that space travelers, especially those performing extravehicular tasks may be exposed to HZE particles in virtually all organs of the body (Todd, 1983). It has been known for several years now that these particles can produce some significant effects on motor behavior. As examples Philpott and colleagues (1985) have shown that mice given brief exposure to low doses of ^{40}Ar particles showed time-dependent reductions in performance of a wire suspension task. More recently, we have shown similar effects on wire suspension task in animals exposed to low doses of either 600 MeV or 1GeV/ nucleon ^{56}Fe irradiation (Joseph et al., 1992). If we can extrapolate from these studies, as

well as those in which organisms were exposed to other radiation sources and motor deficits were observed (Joseph et al., unpublished), one might postulate that either delayed or immediate (perhaps mission compromising) motor deficits might occur on long space explorations such as a Mars expedition.

The precise central locus(i) of these deficits is still unknown. However, studies in non-irradiated organisms suggest that the nigrostriatal system may be important in mediating these effects. The striatum (the end terminus of this system) is one of the basic central processing areas involved in mediating motor behavior. This structure appears to control a variety of motor responses ranging from the simple, (balance and coordination, e.g., Bernheimer, et al., 1973) to the complex, (the ordering and sequencing of intricate behavioral patterns directed by exteroceptive stimuli, Cools et al., 1984). It appears that ^{56}Fe irradiation produces two types of effects in the nigrostriatal system which could ultimately be expressed as decrements in motor behavior -- cell loss and deficits in signal transduction and cell loss in the striatum. These are discussed in more detail below.

HZE-EFFECTS ON STRIATAL NEURONAL FUNCTION

Radiation-induced Alterations in Cell Signaling:

Studies from our laboratory conducted over the last several years have examined organisms exposed to ionizing radiation and assessed specific signal transduction changes in the muscarinic receptors that utilize the phosphoinositide system. Neurotransmitters and other ligands relay transfer messages through receptors such as the muscarinic receptors. These receptors are linked to signal-transducing guanine regulatory binding proteins (G proteins; See reviews by Ali and Agrawal, 1994; Axelrod, et al., 1988; Birnbaumer, 1991;; Raymond, 1995; Rens-Domiano and Hamm, 1995). G proteins are heterotrimers composed of α, ß, γ subunits. Upon stimulation, guanine diphosphate (GDP) is replaced with guanine triphosphate (GTP) and the G protein becomes dissociated from the receptor (Specifically, the α subunit becomes dissociated from the ßγ subunits). It is the α subunits which activate specific second messengers that further convey the signals to other neurons or subsequent targets. Following stimulation, the receptor G-protein complex reassociates when an enzyme, GTPase, hydrolyzes GTP to GDP, which causes the α subunit to reassociate with the ßγ subunits. During stimulation, there are a great number of second messengers that can be activated, depending upon the particular receptor G-protein complex that is stimulated. In the family of the muscarinic receptors subtypes M1-M5. Stimulation of the M1 and M3 subtypes initiate phosphoinisitol hydrolysis wherein phosphatidylinositol (PI e.g., Berridge and Irvine, 1989; Fisher and Agranoff, 1987; for reviews) is phosphorylated to form phosphatidylinositol 4-phosphate (PIP) which is further phosphorylated to form phosphatidylinositol 4,5 bisphosphosphate (PIP2) which is further broken down under the actions of phospholipase C to 1,4,5-inositoltrisphosphate (IP_3) and diacylglycerol (DAG), two of the second messengers in this system IP_3 acts to evoke the quantal release of Ca^{2+} from internal stores (see also Rooney and Thomas, 1993; Tsunoda, 1993). While this is a greatly oversimplified summary, it should be clear that radiation-induced decrements at any point in this process would result in reduced responsiveness to agonist stimulation (i.e., loss of receptor sensitivity) and consequently, behavioral deficits.

Thus, studies have shown that superfused striatal slices obtained from young (3 mo) rats exposed to low doses (0.1 Gy, 1 Gy = 100 rads) of ^{56}Fe irradiation (600 MeV or 1 GeV) exhibited reductions in the ability of oxotremorine, a potent muscarinic agonist, to enhance K^+- evoked dopamine release (K^+-ERDA) (Joseph et al., 1992). These animals had also previously exhibited motor behavioral decrements. The alterations were similar to those seen in aged animals. These (K^+-ERDA) decrements were evident as long as 180 days after irradiation. Subsequent experiments, using similar procedures as described above, indicated that in addition to these declines, there were reductions in carbachol-stimulated 1-4-5-inositol trisphosphate activity, the primary second messenger in this pathway (Joseph et al., 1993) and carbachol-stimulated low K_M GTPase

activity (Villalobos-Molina, et al., 1994), as mentioned above, an indicator of receptor-G protein coupling/uncoupling. Conversely, when the muscarinic receptor signal transduction pathway was "bypassed" and the calcium ionophore A23187 utilized to enhance K^+-ERDA (Joseph et al., 1993), no deficits were observed. Deficits could also be reduced by activating both cyclic AMP and PI pathways simultaneously (Joseph et al., 1994).

Thus, it might be suggested from these findings and those discussed above that the one of the primary sites of oxidative damage in radiation is in the membranes containing the mAChR complex. It is known that lipid peroxide can alter membrane structure and function by several methods (Schroeder, 1984). Numerous experiments have suggested that radiation may modify the transport mechanisms being activated by free radicals or by responding to increased lipid peroxide (Stark, 1991). In addition, as Stark points out in an excellent review (Stark, 1991), certain free radicals are able to gain access into the interior of the membrane and react with the lipid matrix or with membrane-bound proteins, G proteins or even membrane-containing neurotransmitter receptors (e.g., mAChR). Several membrane parameters (e.g., shape, permeability, and osmotic fragility) are determined by membrane phospholipids and membrane fluidity. It is not difficult to see that changes in any of these parameters could alter muscarinic receptor-mediated phosphoinositide signal transduction (ST) in senescence.

Radiation-Induced Increases in Nigral Cell Loss:

One of the more striking changes produced by ^{56}Fe irradiation (0.1-1.0 Gy, 600 MeV) are profound losses in tyrosine hydroxylase immunoreactivity in the substantia nigra. These losses approach 60-80% (depending upon concentration). Interestingly, significant decreases in cell numbers occur in this brain area similar to the losses seen in Parkinson's disease, where profound motor behavioral deficits have been observed. Unfortunately, further examinations of the extent and specificity of this cell loss have been hampered by access to ^{56}Fe-generating facilities. We will, however, attempt to continue these assessments of cell loss at the Brookhaven National Laboratory using accelerated iron particle beams from the Alternating Gradient Synchrotron.

Radiation-Aging Parallels:

One of the more interesting findings from this set of experiments is that the results obtained with respect to signal transduction in these young animals were very similar to those seen as a function of aging. Thus, investigations carried out using superfused slices from old and young animals indicated that there were reductions in oxotremorine-enhanced K^+-ERDA in old animals, indicating that these muscarinic receptors are showing deficits in signal transduction that are contributing to their loss of sensitivity (e.g., see Joseph et al., 1988a, Joseph and Roth, 1991; Roth, et al., 1995). These deficits were associated with reduced IP_3 formation following stimulation (Joseph et al., 1990) and a reduction in IP_3 Ca^{2+} mobilization efficiency from cortical microsomes of old rats (Burnett et al., 1990), even though there is no loss in density or affinity of IP_3 receptors in the cortex and cerebellum (W.S. Pou and E.E. El-Fakahany, unpublished observations) or in the striatum (Joseph et al., 1990).

Just as seen with radiation, studies have indicated that whenever the ligand-mAChR-G-protein interfaces are "bypassed", the age-related decrements in signal transduction are reduced, suggesting that the deficits occur early in the process. As an example, no age differences in enhancement were observed following application of IP_3 or the Ca^{2+} ionophore, A23187 to the striatal slices to directly enhance K+-ERDA indicated (Joseph et al., 1988b). Subsequent investigations have also shown that there are age-related reductions in muscarinic agonist stimulated GTPase activity from hippocampus and striatum (Yamagami et al., 1992). The finding that it does not increase in old animals following stimulation indicates that the deficit may at the receptor-G protein

interface.

In summary it appears that the signal transduction deficits following HZE radiation exposure or in senescence, may result in decreases in sensitivity in muscarinic receptors. These deficits may have their locus within the membranes in which these receptors are contained. Alterations in the microenvironments, created by a decreased ability to respond to oxidative stress that has been observed in aging (e.g., see Joseph et al., 1996) or oxidative stress induced by radiation, can have profound effects on receptor function. Since these receptors are important in mediating both cognitive and motor function these changes could be expressed as behavioral deficits.

One other important consideration here is that neither of these factors (i.e., aging and radiation) can be considered as a single entity. It is almost certain that at least some subset of those who are chosen for long-term space flights will be middle aged or approaching middle age. Therefore, the HZE's would be impinging upon brain areas that are already experiencing the effects of aging, and the effects may be additive or synergistic. For example, as mentioned above, the oxidative stress induced by HZE's would have profound membrane altering effects. In aging there are significant changes in membrane constituencies that include increased: cholesterol/phospholipid ratios and sphingomyelin content and rigidity. Recent observations from our laboratory indicate that increasing sphingomyelin content in PC-12 cells profoundly decreases the ability of the cell to respond to an oxidative stressor such as hydrogen peroxide.

In fact, experiments have shown that experimental alterations in membrane microenvironments can have effects on receptor sensitivity even in the absence of oxidative stress. Joseph et al. (1995) have suggested that there may be decreases in membrane fluidity in senescence which alters the ability of the mAChR-G protein complex to couple/uncouple. In this experiment, incubating striatal tissue in S-adenosyl methionine, a potent membrane fluidizing and phospholipid methylating agent, for one hour prior to stimulation increased muscarinic enhanced K+-ERDA from striatal slices from old animals. Conversely, incubation of striatal tissue from young animals in cholesterol, which increases membrane viscosity, for one hour prior to stimulation reduced K+-ERDA to a level seen in the tissue in old, untreated animals. Exposure to radiation may therefore worsen an already changing situation. It is critical, then, to examine the effects of HZE's in middle aged or older animals in order to determine aging-radiation interactions on membrane microenvironments and subsequent signal transduction.

It is also important to mention here that similar signal transduction deficits may be seen in Alzheimer's disease and may be responsible for the reduced efficacy in cholinergic replacement therapy (see Cutler et al., 1994; Flynn et al., 1995; Fowler et al., 1990; Fowler et al., 1995; Roth, et al., 1995 for reviews). This research has indicated: a 40% reduction in PI levels in the anterior temporal cortex (Stokes and Hawthorne, 1989; see also Jope et al., 1994), a 70% loss of IP_3 binding in temporal cortex and hippocampus (Young et al., 1988); and a decrease in the number of high affinity binding sites (mAChR that are coupled to G proteins are in a high affinity state; when they become uncoupled they are converted to a low affinity state, Flynn et al., 1991). One question that could arise here is that could HZE particle exposure in middle-aged individuals genetically predisposed to age-related neurodegenerative disorders such as Alzheimer's or Parkinson's diseases (where there is also a increased vulnerability to oxidative stress) induce the premature appearance of these diseases? Would such manifestations be mission compromising, or would they be delayed until the return to earth?

Modification of HZE Effects:

While there is certainly a great deal of effort that is being expended to address the issues of shielding on long-term space flights, the question may be asked as to other measures that might be utilize to more clearly reduce the effects of the HZE's. We are examining a host of membrane modification techniques to oxidative stress as well as other variables relevant to aging that contribute to oxidative stress vulnerability and which could

be applied to animal and cellular experiments using HZE's. However, one other area that has not received much attention in this regard is in the area of nutrition. The question could be asked as to whether diet modification may reduce the vulnerability to HZE's on long term space flights. Questions concerning nutrition and aging have become of paramount importance in recent years, and since some of the same mechanisms may be involved in both aging and radiation, it may be that nutritional intervention for the retardation of the deleterious effects of aging may be effective as protection against some of the CNS effects of HZE's.

As an example, consider the results of some studies showing the central effects of consumption of various flavonoid compounds. Considerable literature was found on an extract (Often referred to as EGb 761) from the leaves of Ginkgo biloba (maidenhair tree). The most important substances found in this extract are flavonoids and terpenoids. Flavone glycosides account for 24% of the substances in the extract with the glycosides of kaempherol, quercetin, myricetin and isorhamnetin with glucose or rhamnose being the most important. Several studies have been completed in human subjects consuming this extract and a generally positive conclusion was obtained from over 10 independent experiments. Consumption of 30 mg per day of a mixture of flavonoid glycosides in thirty-one human subjects over the age of 50 years who exhibited a mild to moderate degree of memory impairment in a 6-month double-blind placebo controlled experimental design resulted in a beneficial effect on cognitive function (Rai et al., 1991). Symptoms of "cerebral insufficiency" which have been improved by consumption of this extract of flavonoids include: difficulties of concentration and of memory; absent mindedness; confusion; lack of energy; tiredness; decreased physical performance; depressive mood; anxiety; dizziness; tinnitus; and headache (Kleijnen and Knipschild, 1992ab).

In animals studies using the flavonoids from Ginkgo biloba, myricetin and quercetin, which are two of the predominant glycosides, both decreased the Ca^{+2} - induced increase in the oxidative metabolism of brain neurons. (Oyama et al., 1993). Consumption of the flavonoid glycosides from EGb 761 for 7 months in 15 mo. old mice significantly increased the projection field of infrapyramidal mossy fibers in the hilus region of the hippocampus and significantly reduced the area of the stratum radiatum (Barkats et al., 1994). These authors suggested that the antioxidant properties of these flavonoid glycosides may explain their neuroprotective and neurotropic improvements in memory and other cognitive functions in both humans and experimental animals. In 24-mo. old Wistar rats, chronic administration of the flavonoid glycosides from EGb 761 restored age related declines in receptor density in cerebral cortex membranes for 5-hydroxytryptamine (Huguet et al., 1994). Oral administration of EGb 761 for 4 or 8 weeks to mice in an appetitive operant conditioning experiment facilitated memory processes including improvement in the retrieval of a learned response (Winter, 1991). Thus, from the available evidence it might be postulated that flavonoid glycosides can: 1) improve cerebral metabolism, 2) protect the brain against hypoxic damage and 3) scavenge free-radicals.

Taken together, the results reviewed above suggest that some of the age-induced changes at the receptor level may translate into the observed deficits in memory and motor performance, but dietary manipulations might be a method to decrease the deleterious effects of aging on neuronal function. Extending this to HZE exposure in space it might be reasonable to ask whether some protection against such exposure could be accomplished nutritionally? Could these travelers take similar compounds before the mission and also during the flight to reduce the effects of HZE's. These are clearly considerations that should be addressed by NASA. It may be that the "final frontier" may be one place where the old saying concerning, "You are what you eat." becomes more true than ever before.

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